

5    WHAT IS CLAIMED IS:

1.    A composition, comprising:  
a first component comprising a membrane;  
a cationic membrane permeable dye; and,  
an anionic membrane permeable redistributing dye.
- 10        2.    The composition of claim 1, the cationic membrane permeable dye comprising a cationic nucleic acid staining dye.
3.    The composition of claim 1, wherein the first component comprises one or more of: a cell, a mitochondria, a chloroplast, a cell vesicle, and an artificial membrane.
- 15        4.    The composition of claim 3, wherein the cell comprises one or more of: an intact cell, an animal cell, a plant cell, a fungal cell, a bacterial cell, a mammalian cell, a primate cell, a rodent cell, a canine cell, a feline cell, and a livestock cell, a cultured cell, a THP-1 cell, a COS cell, a CHO cell, a HEK cell, a HeLA cell, an NIH 3T3 cell, a primary cell, an endoderm cell, an ectoderm cell, a mesoderm cell, a cell derived  
20    from a differentiated tissue, a cell derived from an undifferentiated tissue, a blood cell, a peripheral blood cell, a nerve cell, a muscle cell, a skin cell and a bone cell.
- 25        5.    The composition of claim 1, wherein the cationic membrane permeable dye comprises one or more of: a Blue-fluorescent SYTO dye, a Green-fluorescent SYTO Dye, an Orange-fluorescent SYTO dye, a Red-fluorescent SYTO dye, SYTO 62, Pur-1, thiazol, aryl, 2DS-7J1, Hoechst 33258, Hoechst 33342 and hexidium iodide; or wherein the anionic membrane permeable redistributing dye comprises one or more of: an anionic bis-isoxazolone oxonol dye, a bis-oxonol dye, Oxonol V, Oxonol VI, DiBAC<sub>4</sub>(3) DiBAC<sub>4</sub>(5), and DiBAC<sub>2</sub>(3).
- 30        6.    A container or microfluidic processor comprising the composition of claim 1.
7.    A method of generating a signal output which is sensitive to membrane potential, the method comprising:

5           providing a first component comprising one or more membranes;  
          adding a cationic membrane permeable nucleic acid staining dye to the first  
          component; and,  
          monitoring a first signal output from the cationic membrane permeable nucleic  
          acid staining dye, wherein the first signal output is correlated with the transmembrane  
10       potential across the one or more membranes.

          8.    The method of claim 7, the method comprising monitoring a change  
          in transmembrane potential, the monitoring the first signal output comprising monitoring  
          a change in the first signal output over time and the correlating the first signal output with  
          the transmembrane potential across the one or more membrane comprising determining  
15       the rate of change of the first signal over time.

          9.    The method of claim 7, the providing step comprising flowing the  
          first component through a first microfluidic channel, which microfluidic channel  
          intersects a second microfluidic channel, wherein the cationic membrane permeable  
          nucleic acid staining dye is flowed through the first or second microchannel and into  
20       contact with the at least one membrane.

          10.   The method of claim 9, the method comprising flowing an anionic  
          membrane permeable redistributing dye or a neutral dye through the first or second  
          channel and into contact with the at least one membrane.

          11.   The method of claim 7, further comprising exposing the one or more  
25       membranes to one or more transmembrane potential modulatory compositions and  
          monitoring an effect of the one or more transmembrane potential modulatory  
          compositions on the first signal output, thereby monitoring an effect of the one or more  
          transmembrane potential modulatory compositions on the transmembrane potential.

          12.   The method of claim 11, the one or more transmembrane potential  
30       modulatory compositions comprising one or more of: a hyperpolarization buffer, a  
          depolarization buffer, and a compound which alters transport of an ion across the cell  
          membrane.

- 5                   **13.** A method of producing a signal which is dependent on transmembrane potential, the method comprising:
- flowing a first mixture comprising one or more membranes and one or more voltage sensitive dyes through a first channel region; and,
- monitoring at least a first signal output from at least one of the voltage sensitive
- 10 dyes, thereby producing a signal which is dependent on the transmembrane potential across the one or more membranes.
- 14.** The method of claim 13, wherein the voltage sensitive dyes comprise one or more membrane permeable redistributing dyes, which one or more membrane permeable dyes comprise one or more ionic dye and wherein the one or more membrane
- 15 permeable dyes are flowed from a source to the first channel region and into contact with the one or more membranes and wherein flow of the membrane permeable labels across the membrane is detected by monitoring the one or more signal outputs from the membrane permeable labels before an equilibrium distribution is reached.
- 15.** The method of claim 13 or 14, wherein the first mixture comprises
- 20 one or more of: a cationic dye, a cationic membrane permeable nucleic acid staining dye, an anionic dye and a neutral dye.
- 16.** The method of claim 15, wherein the one or more voltage sensitive dyes comprises an anionic dye, a cationic dye, or a cationic membrane permeable nucleic acid staining dye and one or more of: an anionic dye, Oxonol V, Oxonol VI, DiBAC<sub>4</sub>(3),
- 25 DiBAC<sub>4</sub>(5), DiBAC<sub>2</sub>(3), WW781, RGA-30, a cationic dye, an indo-carbocyanine dye with a short alkyl tail, a thio-carbocyanine dye with a short alkyl tail, an oxa-carbocyanine dye with a short alkyl tail, an amino naphthylethylenyl pyridinium dye, a dialkyl amino phenylpolyenyl pyridinium dye, a cationic membrane permeable nucleic acid staining dye, a SYTO dye, SYTO 62 and a neutral dye.
- 17.** The method of claim 13, wherein the first mixture is provided to the
- 30 first channel by flowing a first component comprising one or more membrane from a source to a first channel region and flowing a labeling composition comprising the one or more voltage sensitive dyes into contact with the membrane.

- 5                   **18.** The method of claim 13, further comprising: hyperpolarizing or depolarizing the membrane, or changing a permeability property of the membrane, and monitoring flow of the at least one voltage sensitive dye across the membrane by monitoring the first signal output, thereby measuring changes in the transmembrane potential.
- 10                   **19.** The method of claim 13, further comprising flowing at least a second mixture comprising one or more second voltage sensitive dyes into contact with the membrane and monitoring flow of the one or more second voltage sensitive dyes across the membrane by monitoring at least a second signal output from the second voltage sensitive dyes.
- 15                   **20.** The method of claim 7, 13 or 19, comprising monitoring the first or second signal outputs over a selected period of time (t), which period is less than about 100 seconds.
- 21.** The method of claim 20, wherein t is between about 0.1 and about 80 seconds.
- 20                   **22.** The method of claim 7, 13 or 19, wherein the first or second signal output is monitored at one or more time points, which one or more time points are before equilibration of the first voltage sensitive dyes, the at least second voltage sensitive dyes or the cationic membrane permeable nucleic acid staining dye across the one or more membranes.
- 25                   **23.** The method of claim 13, wherein the voltage sensitive dye is a cationic membrane permeable nucleic acid staining dye.
- 24.** The method of claim 7, 13 or 23, wherein a rate of dye translocation across the membrane depends on the transmembrane potential across the membrane.
- 25.** The method of claim 7 or 23, wherein the cationic membrane permeable nucleic acid staining dye is a cyanine dye, or a cyclic-substituted unsymmetrical cyanine dye.
- 30

- 5                   **26.** The method of claim 7 or 23, wherein the cationic membrane permeable nucleic acid staining dye is a dye selected from: a Blue-fluorescent SYTO dye, a Green-fluorescent SYTO Dye, an Orange-fluorescent SYTO dye, a Red-fluorescent SYTO dye, Pur-1, thiazol, aryl, 2DS-7J1, Hoechst 33258, Hoechst 33342 and hexidium iodide.
- 10                   **27.** The method of claim 7 or 23, wherein the dye is the Red-fluorescent dye SYTO 62.
- 28.** The method of claim 7 or 23, the method comprising adding an anionic membrane permeable redistributing dye to the first component or to the first mixture and measuring a second signal output from the anionic membrane permeable  
15                   redistributing dye, thereby providing a further indication of changes in the transmembrane potential.
- 29.** The method of claim 28, further comprising determining a ratio of the first and second signal.
- 30.** The method of claim 28, wherein the anionic membrane permeable  
20                   redistributing dye comprises one or more of: an anionic bis-isoxazolone oxonol dye, a bis-oxonol dye, Oxonol V, Oxonol VI, DiBAC<sub>4</sub>(3), DiBAC<sub>4</sub>(5), and DiBAC<sub>2</sub>(3).
- 31.** The method of claim 28, wherein the cationic membrane permeable nucleic acid staining dye is SYTO 62, at a concentration of between about 0.01 and about 50  $\mu$ M and the anionic dye is DiBAC<sub>4</sub>(3), at a concentration of between about 0.01 and  
25                   about 50 $\mu$ M.
- 32.** The method of claim 7 or 13, wherein the first membrane is a component of an intact cell, which cell is suspended in a fluid comprising one or more ion selected from: Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, H<sup>+</sup>, Ca<sup>2+</sup>, and HCO<sub>3</sub><sup>-</sup>.
- 33.** The method of claim 7 or 13, wherein the one or more membrane is a  
30                   cell membrane.
- 34.** The method of claim 33, wherein the cell membrane is present in an intact or live cell, or, wherein the cell is selected from: an animal cell, a plant cell, a

5 fungal cell, a bacterial cell, a mammalian cell, a primate cell, a rodent cell, a canine cell, a  
feline cell, a livestock cell, a cultured cell, a THP-1 cell, a COS cell, a CHO cell, a HEK  
cell, a HeLA cell, an NIH 3T3 cell, a primary cell, an endoderm cell, an ectoderm cell, a  
mesoderm cell, a primary cell derived from differentiated tissue, a primary cell derived  
10 from undifferentiated tissue, a primary cell derived from blood, a primary cell derived  
from peripheral blood, a primary cell derived from nerve, a primary cell derived from  
muscle, a primary cell derived from skin and a primary cell derived from bone.

35. The method of claim 34, wherein the intact or live cell has a  
transmembrane potential of about  $-100\text{mV}$  to about  $10\text{mV}$ .

36. The method of claim 7 or 13, wherein the first signal is detected  
15 optically.

37. The method of claim 7 or 13, wherein the first signal is detected at  
between about  $20^{\circ}\text{C}$  and  $40^{\circ}\text{C}$ .

38. The method of claim 7 or 13, wherein monitoring the first signal  
output comprises detecting one or more fluorescent emission produced by the cationic  
20 membrane permeable nucleic acid staining dye or the membrane permeable label.

39. The method of claim 7 or 13, further comprising contacting the first  
component with a transmembrane potential modulator and monitoring an effect of the  
transmembrane potential modulator by monitoring the first signal output.

40. The method of claim 39, wherein the transmembrane potential  
25 modulator is a control modulator or a test modulator.

41. The method of claim 40, wherein the control modulator is selected  
from: a molecule, a neurotoxin, a set of neurotoxins, a neurotransmitter, a set of  
neurotransmitters, a protein, a set of proteins, a peptide, a set of peptides, a lipid, a set of  
lipids, a carbohydrate, a set of carbohydrates, an organic molecule, a set of organic  
30 molecules, a drug, a set of drugs, a receptor ligand, a set of receptor ligands, an antibody,  
a set of antibodies, a cytokine, a set of cytokines, a chemokine, a set of chemokines, a

5 hormone, a set of hormones, a cell, a set of cells, a protein attached to a cell, and a protein attached to a set of cells.

42. A method of generating an optical signal which is sensitive to transmembrane potential, the method comprising:

providing a first component comprising one or more membrane;  
10 adding a cationic membrane permeable redistributing dye to the first component;  
adding an anionic membrane permeable redistributing dye to the first component;  
and,  
measuring a first signal output from the cationic dye and a second signal output  
from the anionic dye, wherein one or more of the first and second signal outputs  
15 comprises an optical signal output, thereby generating the optical signal which is sensitive to the transmembrane potential.

43. The method of claim 42, wherein the cationic dye is a membrane permeable nucleic acid staining dye or a cationic rhodamine, an indo-carbocyanine dye, a thio-carbocyanine dye, an oxa-carbocyanine dye, an amino naphthylethylenyl pyridinium  
20 dye, a dialkyl amino phenyl polyphenyl pyridinium dye, or wherein the anionic membrane permeable redistributing dye comprises one or more of : Oxonol V, Oxonol VI, and DiBAC<sub>4</sub>(3) DiBAC<sub>4</sub>(5), DiBAC<sub>2</sub>(3).

44. The method of claim 42, further comprising adding a neutral dye to the first component.

25 45. The method of claim 42, further comprising adding a neutral dye to the first component, wherein the neutral dye produces a control signal output which is dependent on one or more of: temperature, incubation time and overall membrane permeability.

46. A method of generating an optical signal which is dependent on  
30 transmembrane potential, the method comprising:  
providing a first component comprising one or more membrane;  
adding at least a first membrane permeable redistributing dye to the first component, wherein the first membrane permeable redistributing dye comprises an ion;

5           measuring one or more signal output from the first redistributing dye before an  
equilibrium dye distribution is established, which one or more signal output comprises at  
least one optical signal output, thereby providing the optical signal which is dependent on  
transmembrane potential.

47. The method of claim 46, further comprising correlating the one or  
10   more signal output to a change in transmembrane potential.

48. The method of claim 46, comprising adding at least a second  
membrane permeable redistributing dye to the one or more component and measuring one  
or more signal outputs from the second membrane permeable redistributing dye before an  
equilibrium dye distribution is established.

49. The method of claim 48, wherein the first and second dyes are added  
15   to the first component at approximately the same time and the signal outputs from the  
first and second membrane permeable redistributing dyes are measured at approximately  
the same time.

50. The method of claim 48, wherein the first and second redistributing  
20   dyes comprise an anionic dye and a cationic dye.

51. The method of claim 48 or 50, wherein the first and second  
redistributing dyes comprise one or more of: an anionic dye, a cationic dye, a cationic  
membrane permeable nucleic acid staining dye, and a neutral dye.

52. The method of claim 48, further comprising adding at least a third  
25   membrane permeable redistributing dye to the one or more component and measuring one  
or more signal outputs from the third membrane permeable redistributing dye before an  
equilibrium dye distribution is established.

53. The method of claim 52, wherein the first and second redistributing  
dyes comprise one or more of: an anionic dye and a cationic dye and wherein the third  
30   membrane permeable redistributing dye comprises a neutral dye.



- 5                   **54.** The method of claim 53, wherein the signal output for the neutral dye is correlated to a temperature-dependent change in membrane permeability or a time-dependent change in membrane permeability.
- 55.** The method of claim 51, wherein the cationic dye is a nucleic acid staining dye.
- 10                   **56.** The method of claim 51, wherein the cationic dye comprises a nucleic acid staining dye selected from: a Blue-fluorescent SYTO dye, a Green-fluorescent SYTO Dye, an Orange-fluorescent SYTO dye, a Red-fluorescent SYTO dye, Pur-1, thiazol, aryl, 2DS-7J1, Hoechst 33258, Hoechst 33342 and hexidium iodide or wherein the anionic redistributing dye comprises one or more of Oxonol V, Oxonol VI,  
15   and DiBAC<sub>4</sub>(3) DiBAC<sub>4</sub>(5), DiBAC<sub>2</sub>(3).
- 57.** The method of claim 42, wherein the anionic redistributing dye comprises Syto 62 and the anionic dye comprises DiBAC<sub>4</sub>(3).
- 58.** A microfluidic device for monitoring transmembrane potential, the  
20   microfluidic device comprising:  
                    a body structure having at least one microscale cavity disposed therein;  
                    a target source of a first composition comprising at least one membrane, which target source is fluidly coupled to the at least one microscale cavity; and,  
                    a source of one or more voltage sensitive dyes which source is fluidly coupled to  
25   the at least one microscale cavity, wherein, during operation of the device, the first composition is contacted to the one or more voltage sensitive dyes in the at least one microscale channel.
- 59.** The device of claim 58, wherein the one or more voltage sensitive dyes comprise one or more of: a cationic membrane permeable staining dye source  
30   comprising one or more cationic membrane permeable dyes, which cationic membrane permeable dye source is fluidly coupled to the at least one microscale cavity, and an anionic membrane permeable redistributing dye source comprising one or more anionic

5 redistributing dye, which anionic redistributing dye source is fluidly coupled to the at least one microscale cavity.

60. The device of claim 58, wherein the cationic dye source is a nucleic acid staining dye.

10 61. The device of claim 58, wherein the device comprises both the cationic membrane permeable staining dye source and the anionic membrane permeable redistributing dye source, and wherein, during operation of the device, the first composition is contacted, in the presence of the cationic membrane permeable dye and the anionic membrane permeable redistributing dye, to at least one transmembrane potential modulatory composition.

15 62. The device of claim 58, the device further comprising a source of at least one potential transmembrane potential modulatory composition, which source is fluidly coupled to the at least one microscale cavity.

20 63. The device of claim 58, further comprising a source of at least one transmembrane potential modulatory composition, wherein, during operation of the device, at least one transmembrane potential modulatory composition is contacted to one or more of: the first composition, the cationic membrane permeable dye, or the anionic membrane permeable redistributing dye.

64. The device of claim 58, the device further comprising a plurality of sources of at least one potential membrane modulatory composition.

25 65. The device of claim 64, wherein the plurality of sources comprise one or more microtiter trays, each of the one or more trays comprising at least one potential membrane modulatory composition.

30 66. The device of claim 65, wherein the microtiter trays are movably mounted proximal to the body structure, wherein the body structure comprises one or more pipettor channels which are structurally configured to access the trays, which one or more pipettor channels are fluidly coupled to the at least one microscale cavity.

5                   **67.** The device of claim 62, wherein the at least one potential membrane modulatory composition comprises one or more of: a membrane hyperpolarization buffer, a membrane depolarization buffer, a compound which alters membrane permeability and a compound which alters transport of an ion across the cell membrane.

10                   **68.** The device of claim 58, further comprising a signal detector located proximal to or within the microscale cavity, which signal detector detects the a signal.

**69.** The device of claim 68, wherein the detector detects the detectable signal for a selected length of time (t), or a selected time point ( $t_p$ ).

**70.** The device of claim 58, wherein the at least one microscale cavity is a first microscale channel, and wherein, during operation of the device:  
15                   the first composition comprising the at least one membrane is flowed from the target source into the microchannel;  
                  the cationic membrane permeable dye or the an anionic membrane permeable redistributing dye is flowed into contact with the first composition; and,  
                  the detectable signal is monitored at one or more selected time points after contact  
20                   of the first composition with the cationic membrane permeable dye or the an anionic membrane permeable redistributing dye.

**71.** The device of claim 70, wherein at least one potential membrane modulatory composition is flowed from the target source into contact with the first composition.

25                   **72.** The device of claim 58 wherein the membrane is a component of an intact cell.